--74. (New) Isolated nucleic acid that includes a sequence of nucleotides encoding an alpha3 subunit of a human neuronal nicotinic acetylcholine receptor and hybridizes under conditions of high stringency to a sequence of nucleotides in the coding sequence of HnAChRα3 deposited under ATCC Accession No. 68278.--

--75. (New) Isolated nucleic acid that includes a sequence of nucleotides encoding a beta2 subunit of a human neuronal nicotinic acetylcholine receptor and hybridizes under conditions of high stringency to a sequence of nucleotides in the coding sequence of HnAChRß2 deposited under ATCC Accession No. 68279.--

REMARKS

In accordance with the present invention, there are provided isolated nucleic acids encoding human neuronal nicotinic acetylcholine receptor subunits, nucleic acids capable of hybridizing thereto, nucleic acid probes derived therefrom, isolated mRNA complementary thereto, transformed cells capable of expressing the protein products encoded by the invention nucleic acids, as well as the proteins encoded by the nucleic acids of the present invention.

By the present Communication, the specification has been amended pursuant to 37 C.F.R. §1.78(A) to contain a specific reference to the parent applications from which the present application claims priority. In addition, claims 53, 57, 58, 62 and 68 have been amended and new claims 73-75 have been added to define Applicants' invention with greater particularity. Claims

64, 65 and 69 have been canceled without prejudice. No new matter is introduced by the amendments, as the amended claim language is fully supported by the specification and claims as originally filed.

The amendments to claims 53, 57, 58 and 68 were made in response to the Examiner's objection with respect to the use of allegedly improper Markush terminology.

Claims 53-63, 66-68 and 70-72 are currently under examination.

I. REJECTIONS AND OBJECTION UNDER 35 U.S.C. § 112

Claims 53-72 stand rejected and the specification stands objected to under 35 U.S.C. § 112, first paragraph, as allegedly failing to provide an enabling disclosure for the production of a DNA encoding a human nNAChR subunit or a purified protein encoded thereby. Additionally, claims 55, 56, and 71 are separately rejected and the specification stands further objected to under 35 U.S.C. § 112, first paragraph, as allegedly failing to provide an enabling disclosure for the production of a substantially pure subunit of a human nNAChR. These rejections/objections are respectfully traversed.

Applicants respectfully disagree with the Examiner's assertion, set forth on page 3, lines 10-13 of Paper No. 12, that the instant specification allegedly fails to

> provide an enabling disclosure for the production of a DNA encoding a human neuronal nicotinic acetylcholine receptor subunit or a purified protein encoded thereby.

Applicants similarly disagree with the Examiner's assertion, set forth on page 4, paragraph 6 of Paper No. 12, that Applicants' specification allegedly fails to

> provide an enabling disclosure for the production of a substantially pure subunit of a human neuronal nicotinic acetylcholine receptor.

The preparation and screening of cDNA libraries, as well as the isolation of DNA clones therefrom are **basic** skills commonly utilized by persons of ordinary skill in the art of molecular biology. The identification of the specific source of neuronal tissue for the generation of cDNA libraries[1] renders construction of such libraries straightforward. Furthermore, at the time of the present invention, cloning kits and packaging systems were commercially available from numerous sources (e.g., Stratagene, Promega, New England Biolabs, Inc., etc.).

Similarly, methods of designing and preparing nucleic acid probes for homology screening were well known and commonly utilized by skilled artisans. Applicants have provided sequence information, and restriction maps of DNAs encoding invention **human** nNAChR subunits, as well as properly depositing such DNA in the form of plasmids with the ATCC pursuant to the terms of the Budapest Treaty. Directed to skilled artisans, the information and guidance set forth in the instant specification, in particular sequence identification and quantitation of regions of

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[1]. See, for example, page 13, lines 23-28 of Applicants' specification. Human cDNA libraries were constructed from neuronal tissues such as pre-frontal cortex, parietal, temporal cortex, brain stem, basal ganglia, and spinal cord.

subunit homology[2], is clearly ample such that any person of skill in the art could readily design and synthesize subunit probes and, in addition, determine optimal stringency conditions for hybridization (based upon the sequences set forth in the specification).

Invention subunits can readily be produced using various methods well known to skilled artisans. An example of a commonly employed means (of molecular biologists) to produce invention subunit(s) and/or functional receptors is to express nucleic acids encoding invention subunits in a suitable host cell. Applicants' specification describes various host cell systems in a manner such that those of skill in the art can readily identify host cells suitable for use in the present invention. Invention host cells are well-known and widely used by molecular and cellular biologists. Indeed, such host cell systems are commercially available. Accordingly, Applicants need not burden the specification with that which is well-known in the art.

For example, Applicants describe and exemplify suitable vectors for introducing invention nucleic acids into host cells. Well-known methods (such as transfection or microinjection) for introducing invention nucleic acids into bacterial, yeast, mammalian and *Xenopus* oocytes are provided throughout the specification. Applicants teach preferred host cells for

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[2]. See, for example, Figures 1-3 (restriction map comparison), Figures 4-6 (restriction map homologies), and Figures 7-9 (sequence homologies).

introducing invention nucleic acids on page 14.[3] Further, Applicants teach well-known methods for introducing exogenous genetic material into a range of well-characterized host cells, such that any person of skill in the art could readily and successfully transform host cells with expression vectors containing invention nucleic acids. Thus, the transformed host cells will transcribe the exogenous nucleic acids of the invention into mRNA, and translate such mRNA into invention subunit(s). A skilled artisan using basic techniques can directly isolate the "expression product" from cells that have been transformed with such expression vectors.

The instant specification is directed to those skilled in the art to which the invention "pertains", and thus need not include that which is common and well known in the art.

> A patent need not teach, and preferably omits what is well known in the art.

Spectra-Physics, Inc. v. Coherent, Inc., 3 USPQ2d 1737, 1743 (Fed. Cir. 1987).

> What is conventional knowledge will be read into the disclosure.

In re Howarth, 210 USPQ 689, 691 (CCPA 1981).

> It is well settled that the disclosure of an application embraces not only what is expressly set forth in words or drawings, but what would be understood by persons skilled in the art. As was said in *Webster Loom Co. v. Higgins et al.*, 105 U.S. 580, 586, the applicant 'may begin at the point where his

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[3]. Preferred eukaryotic cells include, for example, human, rat and mouse cells. Preferred amphibian cells include, for example, *Xenopus laevis* oocytes. Examples of yeast cells include, for example, *Saccharomyces cerevisiae, Pichia pastoris, Candida tropicalis* and *Hansenula polymorpha*. Preferred bacterial (prokaryotic) cells include, for example, *Escherichia coli*.

> invention begins, and describe what he has made that is new and what it replaced of the old. That which is common and well known is as if it were written out in the patent. . .'

In re Folkers and Shunk, 145 USPQ 390, 394 (CCPA 1965) (quoting In re Chilowsky, 108 USPQ 321, 324 (CCPA 1956)).

In all instances described hereinabove, Applicants provide substantial enablement to the skilled artisan for the practice of the present invention. Applicants respectfully submit, therefore, that the claims under examination are, indeed, enabled in the specification such as to allow one of ordinary skill in the art, using well-known techniques, to make **and** use the claimed invention without undue experimentation.

In response to the Examiner's concerns regarding the ATCC deposit, attached hereto, as Exhibit A, is a true copy of the acknowledgment of receipt of invention cell lines issued by the American Type Culture Collection and a declaration by Applicants' Manager of Intellectual Property submitting that the biological deposits set forth on pages 11-12 (i.e., ATCC Accession Nos. 68277, 68278, and 68279) of the subject application have been deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, pursuant to and in satisfaction of the Budapest Treaty on the International Recognition of Microorganisms for Purposes of Patent Procedure and, further, that all restrictions upon the availability to the public of the aforementioned deposited material will be irrevocably removed upon the issuance of a patent from the subject application.

Claims 58 and 62 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. This rejection is respectfully traversed.

The Examiner's concern with respect to the absence of antecedent basis for "the" invention subunits in claim 58 has been rendered moot by the amendment of claim 58 provided herewith. Thus, claim 58 has been amended to require "an" invention human neuronal nicotinic acetylcholine receptor subunit. Additionally, the Examiner's concern with respect to the omission of "are" in claim 62 has been rendered moot by the amendment of claim 62 submitted herewith. Claim 62, as amended, now requires cells of claim 59, wherein said cells **are** eukaryotic cells.

In view of the above amendments and remarks, reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, is respectfully requested.

II. REJECTION UNDER 35 U.S.C. § 102(a)

Claims 53, 54, 57-60, and 63 stand rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Fornasari et al., *Neuroscience Letters* 111:351-356 (1990). This rejection is respectfully traversed.

Fornasari et al. is not a proper reference under 35 U.S.C. §§ 102 or 103. The subject application properly claims priority from International Application No. PCT/US91/02311, filed

03 April 1991, which is a continuation-in-part of USSN 07/504,455, filed April 3, 1990[4]. Applicants are, therefore, entitled to the benefit of the filing date of the predecessor patent applications upon which the subject application is based.

In view of the above remarks, reconsideration and withdrawal of the rejection under 35 U.S.C. § 102(a), is respectfully requested.

III. REJECTIONS UNDER 35 U.S.C. § 103

Claims 53, 54, 57-63, 66-68, and 70-72 stand rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Boulter et al., *Proc. Natl Acad. Sci., USA* 84:7763-7767 (1987) in view of Grenningloh et al., *EMBO J.* 9(3):771-776 (1990), Schofield et al., *FEBS Letters* 244(2):361-364 (1989), and Noda et al., *Nature* 305:818-823 (1983). This rejection is respectfully traversed.

Applicants' invention, as defined by all of the claims, distinguishes over the art by requiring isolated DNA encoding **human** nNACh receptor subunits. Specifically, Applicants' invention, as defined by claims 53, 54, 57 and 58, distinguishes over the art by requiring an isolated or substantially pure nucleic acid molecule encoding a **human** nNAChR subunit (alpha2, alpha3, or beta2).

Applicants' invention, as defined by claims 65-70, distinguishes over the art by providing transformed host cells

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[4]. USSN 07/504,455, filed April 3, 1990, issued November 29, 1994 as U.S. Patent No. 5,369,028.

that express the invention receptor subunit DNAs and RNAs, as well as methods for identifying compounds which bind to invention receptors. Additionally, Applicants' invention, as defined by claim 72, distinguishes over the art by providing a method for making cells having **human** neuronal nicotinic acetylcholine receptor subunits. Applicants' invention, as defined by claim 71, further distinguishes over the art by providing substantially pure **human** nNACh receptors comprising **human** alpha and **human** beta subunits.

In contrast to the instant invention, Boulter et al. do not teach or suggest **human** nNACh receptors or subunits thereof. Similarly, Boulter et al. neither teach nor suggest transformed host cells that express **human** nNACh receptors. Furthermore, Boulter et al. do not teach or suggest methods for identifying compounds which bind to **human** nNACh receptors.

Only Applicants teach isolated **human** nNAChR subunits. Indeed, the existence of homologous **human** nNAChR subunit genes, the nucleotide sequences thereof, or the degree of homology, if any, with the rat nNAChR subunit genes was unknown at the time of the Boulter et al. publication. Where neither the nucleotide sequence of a human [EPO] gene nor its **exact degree of homology** with a monkey [EPO] gene was known at the time of invention, the Court of Appeals for the Federal Circuit has held that

> [w]hile the idea of using the monkey gene to probe for a homologous human gene may have been **obvious to try**, the **realization** of that idea would **not** have been obvious.

Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016, 1023 (Fed. Cir. 1991) (Emphasis added). See also, Fiddes v.

Baird, 30 USPQ2d 1481, 1485 (BPAI 1994) where the Board of Patent Appeals and Interferences similarly found that

> claims which are essentially directed to a DNA molecule encoding **human** basic FGF would **not** have been anticipated or rendered obvious by . . . claims which are directed to a DNA molecule encoding **mammalian** and **bovine** basic FGF.

Id. (Emphasis added).

Applicants' invention is directed to DNA encoding **human** nNAChR subunits. Conversely, Applicants' invention is **not** directed, as implied by the Examiner (page 8, lines 21-25 of Paper No. 14), to methods for isolating the same. Only Applicants define specific human neuronal nNAChR subunits for which there was no prior knowledge as to the nature or degree of differences between nucleic acid sequences encoding nNAChR subunits of other species. Only Applicants, having isolated and characterized the human nNAChR subunits, can teach such differences between nucleic acid sequences encoding nNAChR subunits of other species. Clearly, determination of species differences can be made, with the benefit of hindsight, only **after** the human clone has been characterized. Boulter et al. neither disclose nor suggest human sequences nor in what way such sequences may differ from rat sequences. Such sequence differences can effect significant functional consequences with respect to receptor activity. For example, the human α2 subunit contains a stretch of 23 amino acids (absent in rat α2) within the amino-terminal extracellular domain. Interestingly, the ligand-binding domain is purportedly located in the amino-terminal region and, therefore, sequence differences in this region could potentially effect ligand-binding and, consequently,

ligand-induced receptor activity (see, for example, page 7 of Exhibit B, reporting different agonist sensitivities between rat α2ß2 and human α2ß2). Indeed, it is established, where the prior art fails to teach **any** homology between a mammalian gene and the corresponding human gene, that

> it would have been **highly speculative** for one of ordinary skill in the art to have a reasonable expectation of success in obtaining the . . .gene encoding human . . . by using the . . . gene encoding bovine . . .

Id. at 1485. (Emphasis added). See also, Amgen, *supra* at 18 USPQ2d at 1023 (while it may be obvious to try to probe a human library with a mammalian gene to isolate the corresponding human gene, it is a matter of speculation whether the human gene would be isolated with a reasonable expectation of success). Accordingly, Boulter et al. neither disclose nor suggest Applicants' invention.

The combination of Grenningloh et al., Schofield et al., and Noda et al. is incapable of curing the deficiencies of Boulter et al. The disclosure of inter-species homology among several members of the ligand-gated ion channel superfamily of receptors observed for the glycine, $GABA_A$, and muscle nicotinic acetylcholine receptors is neither relevant to, nor predictive, of any characteristic of the invention receptors.

The superfamily of ligand-gated ion channel receptors comprises various subfamilies, wherein individual members thereof are distinguished from other members based upon such characteristics as, for example, ligand specificity, electrophysiological profile, pharmacological profile and

function. For example, invention receptors having distinct pharmacological and electrophysiological properties are **distinguished**, not only from members of other neurotransmitter receptor subfamilies, but are also distinguished from other receptors within the nNAChR subfamily. See, for example, data provided in the Declaration submitted herewith, as Exhibit B, wherein comparison of rat α2 subunit with human α2 subunit, or comparison of rat α3 subunit with human α3 subunit, or comparison of rat ß2 subunit with human ß2 subunit reveals that the human subunits are separate and distinct from the rat subunits.

Grenningloh et al. do not teach or suggest a human **nNACh** receptor or subunit thereof. Grenningloh et al. disclose human **glycine** receptor subunits. Clearly the glycine receptor is pharmacologically distinct from invention receptors.

Schofield et al. do not teach or suggest a human **nNACh** receptor or subunit thereof. Schofield et al. disclose human **$GABA_A$** receptor subunits. The $GABA_A$ receptor is clearly pharmacologically unrelated to invention receptors.

Noda et al. do not teach or suggest a human **neuronal** acetylcholine receptor. Noda et al. disclose a human **muscle** acetylcholine alpha subunit precursor. The muscle alpha-subunit is **not** capable of forming a functional receptor with the muscle beta-subunit. In fact, muscle acetylcholine receptors (comprising **five** subunits) are stoichiometrically distinct from **neuronal** nicotinic acetylcholine receptors. Although the muscle alpha-subunit is capable of forming a nicotinic acetylcholine receptor, it is not a member of the **neuronal NACh** receptor family

because it exhibits a pharmacological profile separate and distinct from invention receptors.

Contrary to the Examiner's assertion, Applicants respectfully submit that the fourth sentence of the Grenningloh et al. publication, observing that

> [b]y cDNA cloning, subunits of glycine and $GABA_A$ receptors were found to share significant sequence similarity and a conserved transmembrane topology with subunits of the nicotinic acetylcholine receptor, an agonist-gated ion channel

does not teach or suggest the use of the **rat** subunits described by Boulter et al. as probes to screen **human** cDNA libraries. The above-quoted statement from the reference merely proposes a common evolutionary origin for specific structural characteristics that are shared between particular members of the $GABA_A$/glycine receptor subfamily and particular members of the nicotinic acetylcholine receptor subfamily. Grenningloh et al., however, cautions that the similarities referred to are neither shared nor characteristic of other subfamilies (and individual members thereof) comprising the superfamily of ligand-gated ion channels. Indeed, Grenningloh et al. on page 771, col. 2, acknowledge that

> [i]n the case of **neuronal** nicotinic acetylcholine and $GABA_A$ receptors, considerable **heterogeneity** has been shown to exist for individual subunit polypeptides.

(Emphasis added). Thus, Grenningloh et al. clearly do not suggest that such similarities and/or characteristics are, in fact, associated with any other ligand-gated ion channel receptor member of the superfamily.

In summary, the human receptors and/or subunits cited in Grenningloh et al., Schofield et al., or Noda et al. are electrophysiologically, pharmacologically and/or functionally distinct from invention receptors. Moreover, Applicants provide evidence confirming the unique and unpredictable nature of inter-family and inter-species members of the ligand-gated ion channel receptor superfamily (see Exhibit B).

Accordingly, Boulter et al., alone or in combination with Grenningloh et al., Schofield et al., and/or Noda et al. does not obviate the present invention.

The rejection of claims 64, 65 and 69, over the combination of Boulter et al., Grenningloh et al., Schofield et al., and Noda et al. publications as applied to claims 53, 54, 57-53, 66-68, 70 and 72 above, and further in view of Deschamps et al., *Science* 230:1174-1178 (1985) and Greenberg et al., *Science* 234:80-83 (1986) is respectfully traversed.

The Examiner's concerns with respect to the alleged obviousness of claims 64, 65 and 69, over the combination of the above-identified publications has been rendered moot by the cancellation herewith of these claims without prejudice.

In view of the above remarks, reconsideration and withdrawal of the rejections under 35 U.S.C. § 103, is respectfully requested.

IV. SUMMARY

In view of the above amendments and remarks, reconsideration and favorable action on all pending claims is respectfully requested. If any questions or issues remain, the Examiner is invited to contact the undersigned at the telephone number set forth below so that a prompt disposition of this application can be achieved.

Respectfully submitted,

1/27/95
Date

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PRETTY, SCHROEDER,
BRUEGGEMANN & CLARK
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Los Angeles, California 90071

Attachments: ATCC Deposit Receipt and Declaration (Exhibit A)
Declaration Under 37 C.F.R. § 1.132 (Exhibit B)